

Estradiol and Testosterone Regulate Arginine-Vasopressin Expression in SH-SY5Y Human Female Neuroblastoma Cells Through Estrogen Receptors- α and - β

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The expression of arginine-vasopressin (AVP) is regulated by estradiol and testosterone (T) in different neuronal populations by mechanisms that are not yet fully understood. Estrogen receptors (ERs) have been shown to participate in the regulation of AVP neurons by estradiol. In addition, there is evidence of the participation of ER β in the regulation of AVP expression exerted by T via its metabolite 5 α -dihydrotestosterone (5 α -DHT) and its further conversion in the androgen metabolite and ER β ligand 3 β -diol. In this study we have explored the role of ERs in the regulation exerted by estradiol and T on AVP expression, using the human neuroblastoma cell line SH-SY5Y. Estradiol treatment increased AVP mRNA levels in SH-SY5Y cells in comparison with cells treated with vehicle. The stimulatory effect of estradiol on AVP expression was imitated by the ER α agonist 4,4',4',-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol and blocked by the ER antagonist, ICI 182,780, and the ER α antagonist 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1hpyrazoledihydrochloride. In contrast, the ER β agonist 2,3-bis(4-hydroxyphenyl)-propionitrile reduced AVP expression, whereas the ER β antagonist 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol enhanced the action of estradiol on AVP expression. T increased AVP expression in SH-SY5Y cells by a mechanism that was dependent on aromatase but not on 5 α -reductase activity. The T effect was not affected by blocking the androgen receptor, was not imitated by the T metabolite 5 α -DHT, and was blocked by the ER α antagonist 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1hpyrazoledihydrochloride. In contrast, 5 α -DHT had a similar effect as the ER β agonists 2,3-bis(4-hydroxyphenyl)-propionitrile and 3 β -diol, reducing AVP expression. These findings suggest that estradiol and T regulate AVP expression in SH-SY5Y cells through ERs, exerting a stimulatory action via ER α and an inhibitory action via ER β . (**Endocrinology** 154: 2092–2100, 2013)

Arginine-vasopressin (AVP) is expressed by different neuronal populations in the brain (1–3) and is involved in different functions, including the control of water homeostasis (4–7), blood pressure (8–10), thermoregulation (11–13), social behavior (3, 14, 15), and stress response (16–19). Synaptic activity regulates the

firing of magnocellular AVP neurons (20), and in particular approximately 60% of the total number of synapses in the supraoptic (SON) and paraventricular (PVN) nuclei use γ -aminobutyric acid as neurotransmitter (21). In addition, several endocrine factors, including gonadal hormones, may participate in the reg-

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Abbreviations: AR, androgen receptor; AVP, arginine-vasopressin; 5 α -DHT, 5 α -dihydrotestosterone; 3 β -diol, 5 α -androstane-3 β ,17 β -diol; DPN, 2,3-bis(4-hydroxyphenyl)-propionitrile; ER, estrogen receptor; MPP, 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1hpyrazoledihydrochloride; PHTPP, 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol; PPT, 4,4',4',-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol; PVN, paraventricular nucleus; SON, supraoptic nucleus; T, testosterone.

ulation of AVP neurons and AVP expression. Both estradiol and T have been shown to regulate AVP expression in the brain (22–24). In particular, estradiol is able to regulate AVP expression in the PVN of male mice (25), and it increases AVP plasma levels in ovariectomized female rats (22, 26), whereas T regulates AVP expression in the SON (27) and in the bed nucleus of the stria terminalis (28) of male rats.

The mechanisms involved in the regulation of AVP expression by T and estradiol are not fully clarified. Estrogen receptors (ERs) have been shown to participate in the regulation of AVP neurons by estradiol (29–32). ER β is expressed in AVP neurons of the SON and the PVN (33–35), whereas ER α is expressed in the medial preoptic area (31), lateral septum, and medial amygdala (32), important targets for the parvocellular AVP system. Moreover, ER α seems to be involved in the regulation of AVP expression in the SON and PVN of female rats (30, 36), probably through a mechanism mediated by afferent neurons projecting to the SON and PVN and expressing ER α (7, 30, 37, 38). However, there is also evidence of T participation in the ER β regulation of AVP expression via its metabolite 5 α -dihydrotestosterone (5 α -DHT) and its further conversion in 3 β -diol, which is a ligand of ER β (39, 40).

In this study we explored the role of ERs in the regulation exerted by estradiol and T on AVP expression, using the human neuroblastoma cell line SH-SY5Y. These cells express ERs (41) and aromatase (42). In the present study, we determined that SH-SY5Y also expresses AVP and is therefore a good model to assess the role of ERs in the action of T and estradiol on AVP expression. Our findings indicate that both estradiol and T exert stimulatory and inhibitory actions on AVP mRNA levels acting through ER α and ER β , respectively.

Materials and Methods

Cell culture

The human SH-SY5Y neuroblastoma cell line was used in this study. SH-SY5Y cells are genetically female. The line was purchased from American Type Culture Collection (Manassas, Virginia) and was cultured in DMEM-F12 supplemented with 10%

fetal bovine serum. Cells were passaged twice per week and were not used after 10 passages.

Treatments

After 12 hours of serum starvation using serum-free medium, cells were incubated with fresh serum-free medium containing vehicle (ethanol); 17 β -estradiol (from 10⁻¹⁰ to 10⁻⁷ M; Sigma, Madrid, Spain); ICI 182,780 (10⁻⁹ M; Sigma); 4,4',4',-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT; from 10⁻¹⁰ to 10⁻⁷ M; Tocris, Madrid, Spain); 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenyl]-1H-pyrazoledihydrochloride (MPP; from 10⁻⁹ to 10⁻⁶ M; Tocris); 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN; from 10⁻¹⁰ to 10⁻⁷ M; Tocris); 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP; from 10⁻⁹ to 10⁻⁶ M; Tocris); T (from 10⁻⁹ to 10⁻⁵ M; Sigma); 5 α -DHT (from 10⁻¹⁰ to 10⁻⁶ M; Sigma); 3 β -diol (5 α -androstane-3 β ,17 β -diol (from 10⁻⁹ to 10⁻⁶ M; Sigma); flutamide (from 10⁻⁹ to 10⁻⁵ M; Sigma); letrozole (10⁻¹⁰ to 10⁻⁷ M, Novartis, Basel, Switzerland); and/or finasteride (10⁻¹⁰ to 10⁻⁶ M; Sigma).

RNA isolation and quantitative real-time PCR

After 24 hours, cells were lysed and total RNA was extracted using the illustra RNAspin mini-RNA isolation kit (GE Healthcare, Buckinghamshire, United Kingdom) as per the manufacturer's instructions to measure AVP, ER α , and ER β mRNA levels. 18S rRNA was used as the control house-keeping gene. Deoxyribonuclease digestion was carried out on a column using the same kit. The first cDNA strand was synthesized using all RNA extracted by reverse transcription in a final volume of 15 μ L using the RevertAidTM H Minus first-strand cDNA synthesis kit (MBI Fermentas, Bath, United Kingdom) according to the supplied protocol. After reverse transcription, the cDNA was diluted 1:4 and 5 μ L were amplified by real-time PCR in 20 μ L using SYBR Green master mix (Applied Biosystems, Foster City, California) in an ABI Prism 7500 sequence detector (Applied Biosystems), with conventional Applied Biosystems cycling parameters (40 cycles of 95°C, 15 seconds, and 60°C, 1 minute).

Primers for human AVP, human androgen receptor (AR), and human 18S were designed using Primer Express 1.0 (Applied Biosystems) and Amplify software. Primers for human ER α and human ER β were used as previous described in our group (43). See Table 1 for transcript references and primer sequences.

After amplification, the size of the quantitative real-time PCR products was verified by electrophoresis on 2% (wt/vol) ethidium bromide-stained agarose gel, and the identity of PCR products was ascertained by sequencing. Bands were excised and

Table 1. Transcript References and Primer Sequences Used for Quantitative Real-Time-PCR

Transcript	Forward Primer (5'–3')	Reverse Primer (5'–3')
AVP (NM_000490.4)	TGTGTGCACAGGATGCCT	TCAGCTCCAGGTCGGACAT
18S (NR_003286.1)	CGCCGCTAGAGGTGAAATCT	CATTCTTGGCAAATGCTTTTCG
ER α (NM_000125.1)	GCTTCGATGATGGGCTTACTGA	ATCGCGAACCAGATGATGT
ER β *(AF051427)	AGATTCGCCGCTTTGTGG	GCTTCGGCTGCTGTCA
AR transcript 1 (NM_000044.3)	CCCATCCCCACGCTCGCATC	CATGCAGGCTCGCCAGGTCC

cDNA was purified using the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany). One hundred nanograms of AVP, AR, ER α , and ER β mRNA samples were sequenced (Automatic Sequencing Center, Consejo Superior de Investigaciones Científicas, Madrid, Spain) with the same sense and antisense primers. The obtained sequence was aligned with the expected sequence obtained from the GenBank. In addition, dissociation curve analysis was also performed after each PCR to ensure that a single product and no primer-dimers were present. All reactions were performed in triplicate. The relative mRNA expression level was calculated using the $\Delta\Delta CT$ method, using ribosomal 18S RNA as the endogenous RNA control to correct for the amount of total RNA used in each PCR reaction.

Statistical analysis

Data were analyzed via 1-way ANOVA, followed, when appropriate, by a post hoc analysis with the Bonferroni's test. The SPSS 17.0 software (SPSS Inc, Chicago, Illinois) was used for calculating probability values. $P < .05$ was considered statistically significant. Data are represented as the mean \pm SEM. Results of the statistical analyses are shown in the figure legends.

Results

Characterization of AVP, ER, and AR expression in SH-SY5Y neuronal cells

SH-SY5Y cells were initially tested for the expression of AVP, ERs and AR. Real-time PCR indicated the expression of AVP, ER α , and ER β mRNA in this cell line (Figure 1). In contrast, we did not detect AR mRNA in our cells (as positive control we used the prostate adenocarcinoma cell line LNCaP). Sequence analysis by automatic sequencing with the same sense and antisense primers revealed coincidence with human AVP, ER α , ER β , and AR mRNA sequences, respectively.

AVP mRNA levels were up-regulated by estradiol in SH-SY5Y neuronal cells by a mechanism blocked by an ER α antagonist and enhanced by an ER β antagonist

SH-SY5Y cells were treated with either vehicle or estradiol for 24 hours. Estradiol treatment at concentrations of 10^{-7} and 10^{-8} M did not significantly affect AVP mRNA levels. However, a significant increase in AVP mRNA levels was detected when the cells were treated with 10^{-9} or 10^{-10} M estradiol. The maximal effect was observed at 10^{-9} M (Figure 2A). Treatment with ER antagonists MPP, PHTPP, and ICI 182,780 alone, did not affect AVP mRNA levels (data not shown). However, the effect of estradiol was blocked by the ER α antagonist MPP (Figure 2B) and by the ER antagonist ICI 182,780 (Figure 2C) but not by the ER β antagonist

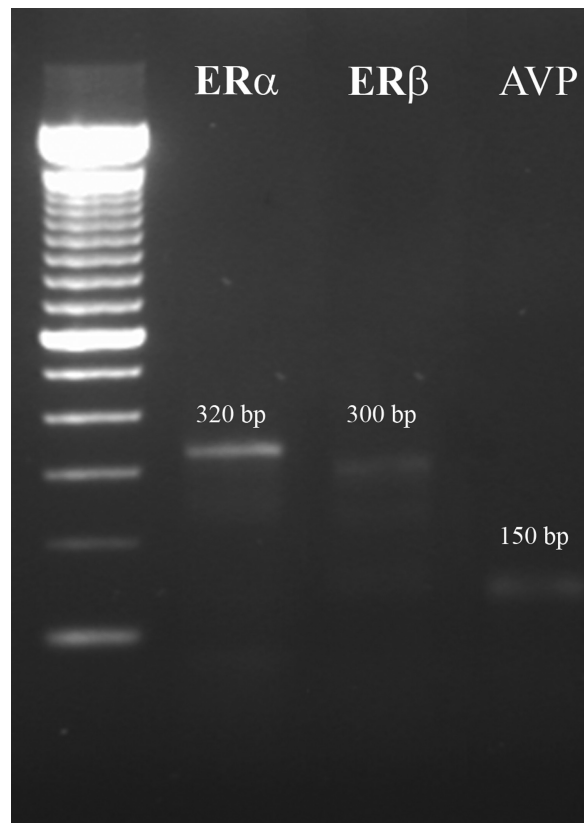


Figure 1. PCR analysis of ER α , ER β , and AVP in SH-SY5Y human neuroblastoma cells.

PHTPP (Figure 2E). In addition, the combination of estradiol with the ER α antagonist MPP at 10^{-7} M resulted in significantly lower AVP mRNA levels compared with control values. In contrast, the combination of estradiol with the ER β antagonist PHTPP at 10^{-7} M resulted in significantly higher AVP mRNA levels compared with estradiol values (Figure 2, B and D). A possible interpretation of these results is that estradiol is acting on both ERs, enhancing or decreasing AVP mRNA levels through ER α or ER β , respectively. To test this hypothesis, in the next experiment, cells were treated with an ER α or an ER β agonist.

AVP mRNA levels were up-regulated by an ER α agonist and down-regulated by an ER β agonist in SH-SY5Y neuronal cells

As shown in Figure 2E, treatment of SH-SY5Y cells with the ER α agonist PPT, at 10^{-8} M, resulted in a significant increase in AVP mRNA levels compared with control values. In contrast, the treatment with the ER β agonist DPN, at 10^{-7} M, resulted in a significant decrease in AVP mRNA levels (Figure 2F). The enhancement of AVP mRNA levels by PPT was stronger than the decrease in

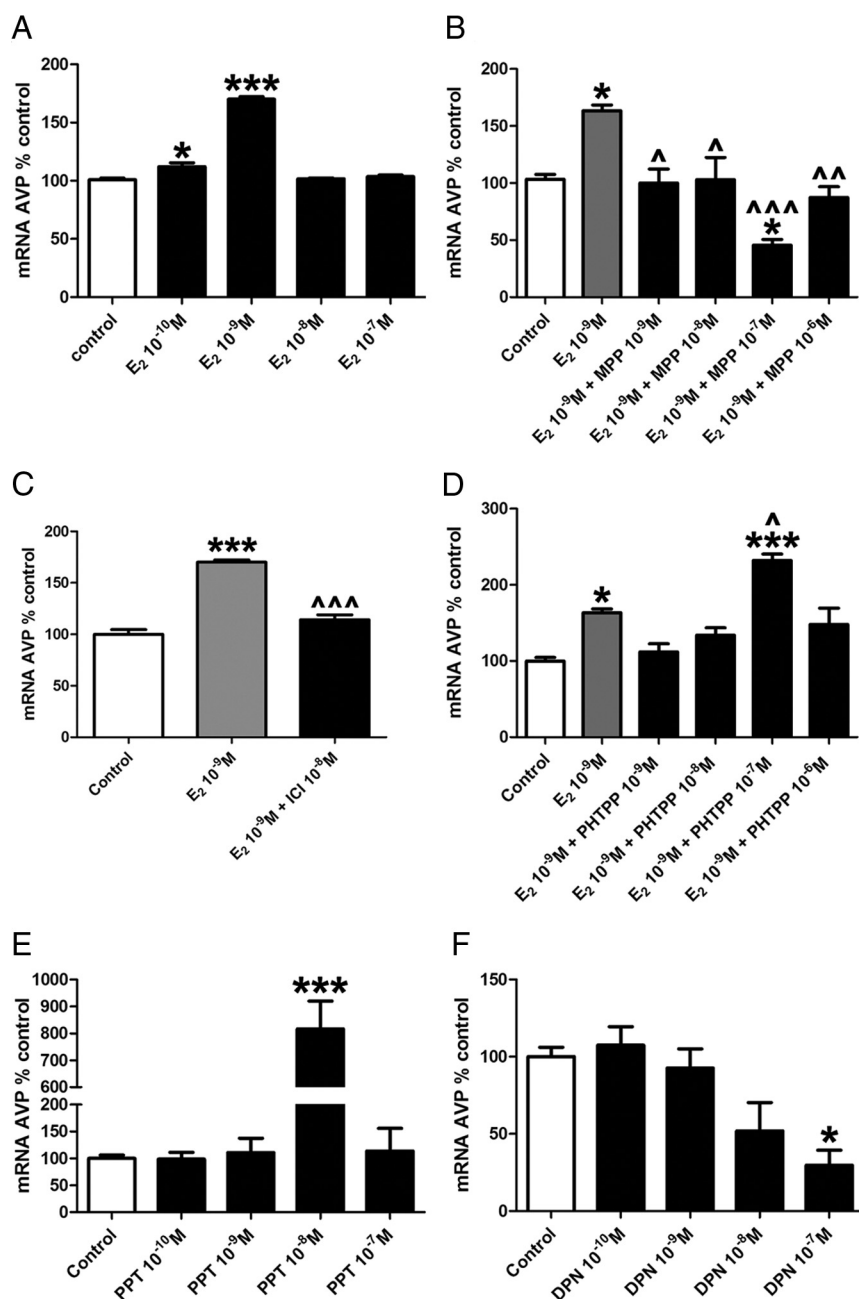


Figure 2. AVP mRNA levels in SH-SY5Y human neuroblastoma cells. A, Cells were treated for 24 hours with vehicle (control) or different doses of 17 β -estradiol (E2). ANOVA: $F_{(4, 19)} = 221.3$; $P < .0001$. B, Cells were treated for 24 hours with vehicle, 10⁻⁹ M E2, or 10⁻⁹ M E2 and different doses of the ER α antagonist MPP. ANOVA: $F_{(5, 17)} = 12.53.3$; $P = .0002$. C, Cells were treated for 24 hours with vehicle, 10⁻⁹ M E2, or 10⁻⁹ M E2 and the ER antagonist ICI 182,780 (ICI; 10⁻⁸ M). ANOVA: $F_{(4, 14)} = 175.9$; $P < .001$. D, Cells were treated for 24 hours with vehicle, 10⁻⁹ M E2, or 10⁻⁹ M E2 and different doses of the ER β antagonist PHTPP. ANOVA: $F_{(5, 17)} = 16.84$; $P < .0001$. E, Cells were treated for 24 hours with vehicle or different doses of the ER α agonist PPT. ANOVA: $F_{(4, 21)} = 25.03$; $P < .0001$. F, Cells were treated for 24 hours with vehicle or different doses of the ER β agonist DPN. ANOVA: $F_{(4, 23)} = 6.78$; $P < .0014$. Data are represented as the mean \pm SEM. Results of the Bonferroni's test: significant differences of *, $P < .05$ and ***, $P < .001$ vs the control value; ^, $P < .05$, ^^, $P < .01$, and ^^, $P < .001$ vs the estradiol value.

AVP mRNA levels caused by DPN. This finding further indicates that ER α and ER β have opposite effects on AVP mRNA levels in SH-SY5Y cells.

AVP mRNA levels in SH-SY5Y neuronal cells were up-regulated by T by a mechanism independent of its conversion to 5 α -DHT and the consequent activation of AR

T, at 10⁻⁸ M, significantly increased AVP mRNA in SH-SY5Y cells compared with control cells (Figure 3A). One possible mechanism of action of the hormone is by its conversion in 5 α -DHT and the activation of AR. Although we did not detect AR mRNA in our cells, previous studies have reported that SH-SY5Y cells metabolize T in 5 α -DHT (44) and express AR (45). Thus, we explored this possible mechanism of regulation. The T-induced AVP mRNA increase was not affected when cells were treated with finasteride, an inhibitor of 5 α -reductase, the enzyme that converts T in 5 α -DHT (Figure 3B). Furthermore, the action of T was not affected when cells were treated with the AR antagonist flutamide (Figure 3C). Finasteride and flutamide alone did not significantly affect AVP mRNA levels (data not shown).

To further exclude the possible implication of AR in the action of T, SH-SY5Y cells were treated with the AR agonist 5 α -DHT. 5 α -DHT treatment, at concentrations ranging from 10⁻⁷ to 10⁻⁹ M, not only did not imitate the effect of T but also even had an opposite effect, resulting in a significant decrease in AVP mRNA levels compared with control values (Figure 3D). The effect of 5 α -DHT was not affected by flutamide (Figure 3E). These findings suggest that T and 5 α -DHT regulate AVP mRNA levels in SH-SY5Y cells in opposite ways and by mechanisms that are independent of AR.

AVP mRNA levels in SH-SY5Y neuronal cells were down-regulated by the 5 α -DHT metabolite 3 β -diol

In the previous experiments, we observed that both 5 α -DHT and the ER β agonist DPN exerted an inhibitory ac-

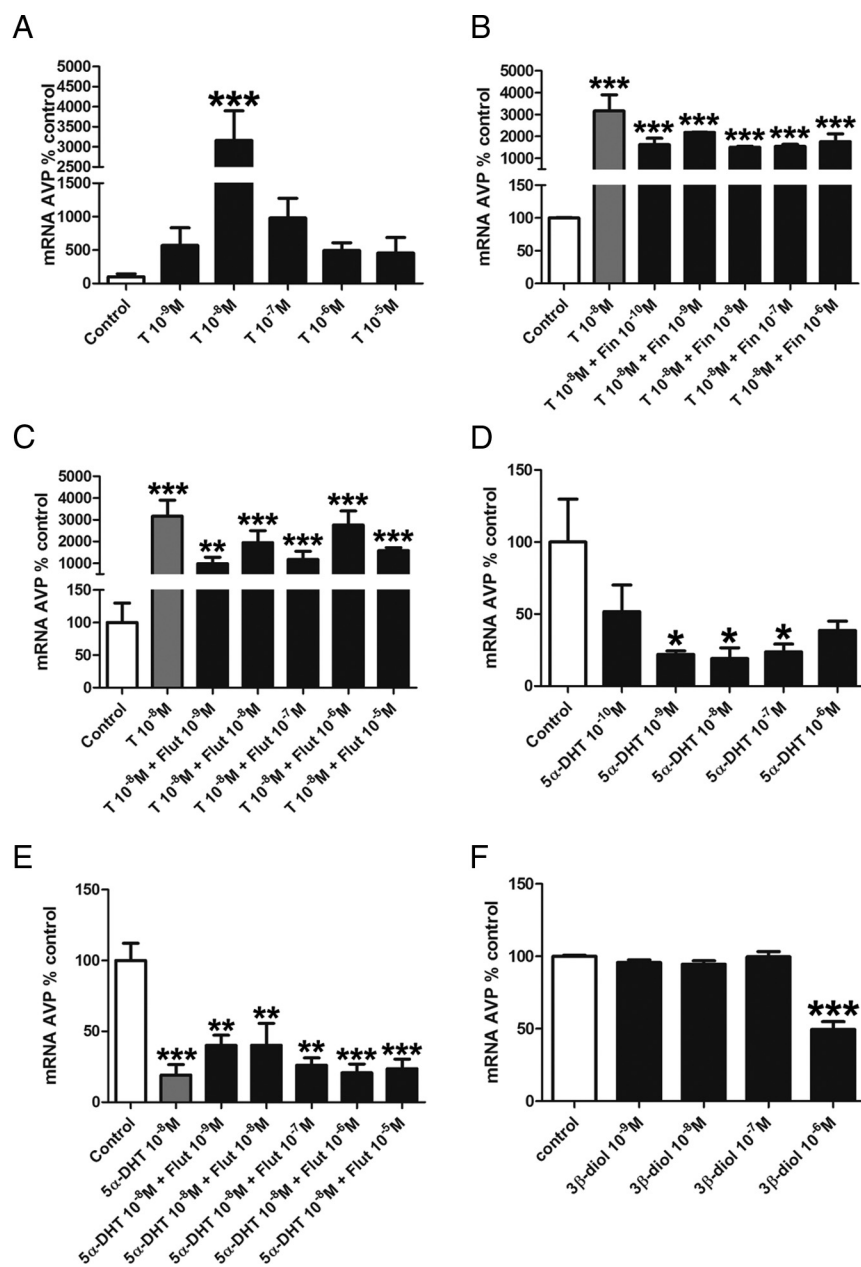


Figure 3. AVP mRNA levels in SH-SY5Y human neuroblastoma cells. A, Cells were treated for 24 hours with vehicle (control) or different doses of T. ANOVA: $F_{(5, 17)} = 9.648$; $P = .0007$. B, Cells were treated for 24 hours with vehicle, 10^{-8} M T, or 10^{-8} M T and different doses of the 5 α -reductase inhibitor finasteride (Fin). ANOVA: $F_{(6, 20)} = 60.14$; $P = .0001$. C, Cells were treated for 24 hours with vehicle, 10^{-8} M T, or 10^{-8} M T and different doses of the androgen receptor antagonist flutamide (Flut). ANOVA: $F_{(6, 20)} = 15.60$; $P < .0001$. D, Cells were treated for 24 hours with vehicle or with different doses of 5 α -DHT. ANOVA: $F_{(5, 18)} = 4.495$; $P < .0134$. E, Cells were treated for 24 hours with vehicle, 10^{-8} M 5 α -DHT, or 10^{-8} M 5 α -DHT and different doses of the androgen receptor antagonist flutamide (Flut). ANOVA: $F_{(6, 21)} = 9.402$; $P < .0002$. F, Cells were treated for 24 hours with vehicle or different doses of the ER β agonist 3 β -diol. ANOVA: $F_{(4, 18)} = 48.18$; $P < .0001$. Data are represented as the mean \pm SEM. Results of the Bonferroni's test: significant differences of *, $P < .05$, **, $P < .01$, and ***, $P < .001$ vs the control value.

tion on AVP mRNA levels in SH-SY5Y cells. Therefore, a possible mechanism for the action of 5 α -DHT is the activation of ER β after its conversion in its metabolite 3 β -diol. Thus, we treated SH-SY5Y cells with 3 β -diol to determine

whether this steroid regulates AVP mRNA levels. 3 β -Diol significantly reduced AVP mRNA levels but only at high concentration (10^{-6} M).

AVP mRNA levels in SH-SY5Y neuronal cells were up-regulated by T by a mechanism blocked by an aromatase inhibitor and an ER α antagonist

We had previously observed that our SH-SY5Y cell line expresses the enzyme aromatase (42), which converts T in estradiol. Therefore, T may potentially affect AVP mRNA levels by the activation of ERs after its conversion into estradiol. To test this hypothesis, SH-SY5Y cells were treated with T in the presence of letrozole, an aromatase inhibitor. Under these conditions, T not only did not increase but also even resulted in a significant decrease in AVP mRNA levels (Figure 4A). This finding suggested that T up-regulates AVP mRNA levels by its conversion in estradiol. To further test this possibility, SH-SY5Y cells were treated with T in the presence or absence of ER antagonists. Interestingly, the up-regulation of AVP mRNA levels by T was blocked by the ER α antagonist MPP and by the ER antagonist ICI 182,780 (Figure 4B). The combination of T with the ER α antagonist MPP resulted in a significant decrease of AVP mRNA levels compared with control values, suggesting a possible inhibitory action of ER β . However, the ER β antagonist PHTPP did not affect the up-regulation of AVP mRNA levels by T (Figure 4B). These findings suggest that T up-regulated AVP mRNA levels in SH-SY5Y cells after its conversion to estradiol and the consequent activation of ER α .

Discussion

AVP is synthesized by different neuronal populations of the central nervous system. AVP synthesized by the mag-

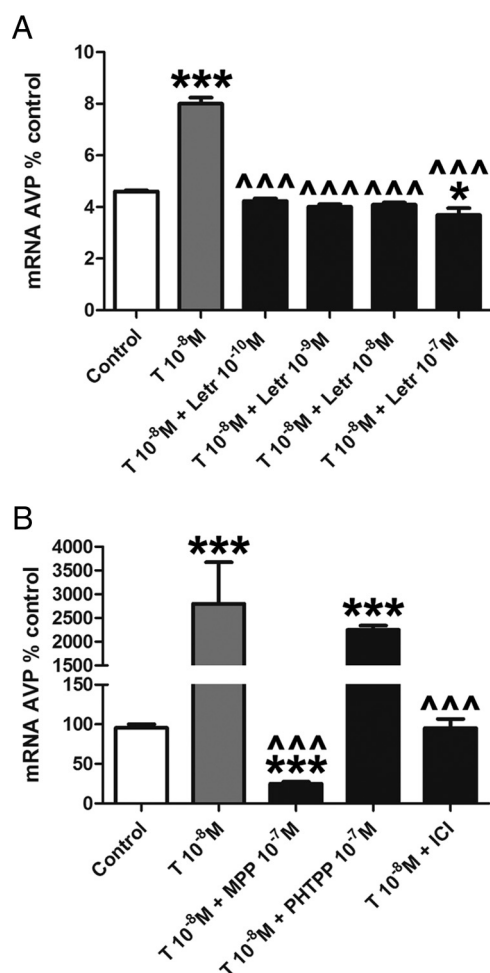


Figure 4. AVP mRNA levels in SH-SY5Y human neuroblastoma cells. A, Cells were treated for 24 hours with vehicle (control), 10^{-8} M T, or 10^{-8} M T and different doses of the aromatase inhibitor letrozole (Letr). ANOVA: $F_{(5, 21)} = 85.76$; $P < .0001$. B, Cells were treated for 24 hours with vehicle, 10^{-8} M T, 10^{-8} M T and 10^{-7} M MPP (ER α antagonist), 10^{-8} M T and 10^{-7} M PHTPP (ER β antagonist), and 10^{-8} M T and 10^{-8} M ICI 182,780 (ICI; ER antagonist). Results of the Bonferroni's test: significant differences of *, $P < .05$ and ***, $P < .001$ vs the control value; ^, $P < .001$ vs the T value.

nocellular neurons of the paraventricular and supraoptic nuclei (46) is released in the neurohypophysis and exert hormonal actions, regulating water balance. AVP synthesized by other neuronal groups, such as the bed nucleus of the stria terminalis, medial amygdala, suprachiasmatic nucleus, and the parvocellular neurons from the paraventricular nucleus (47) act as a neuromodulator and participates in the regulation of circadian rhythms, cardiovascular function, the stress response, social recognition, social behavior, and attachment (1, 48–51). These behaviors and physiological processes are modulated by estradiol and T, which also regulate AVP expression in the brain by mechanisms not fully elucidated. In this study, we have used SH-SY5Y human neuroblastoma cells to assess the role of ERs on the regulation of AVP expression. We

have first shown that SH-SY5Y cells express AVP, ER α , and ER β , therefore being an adequate cellular model for our aims.

Our findings indicate that estradiol increases AVP expression in SH-SY5Y cells. This effect of estradiol was imitated by an ER α agonist, PPT, and was blocked by both an ER antagonist, ICI 182,780, and an ER α antagonist, MPP. In contrast, an ER β agonist, DPN, reduced AVP expression in SH-SY5Y cells. In addition, the ER α antagonist MPP caused a significant reduction in AVP mRNA levels, whereas the ER β antagonist PHTPP enhanced the action of estradiol on AVP expression. These findings suggest that estradiol is acting on both ERs to regulate AVP expression with opposite effects, enhancing AVP expression via ER α and decreasing AVP expression via ER β . The action on ER α predominates at low concentrations of the hormone (10^{-9} to 10^{-10} M), and the result is a net increase in AVP expression after estradiol treatment. However, at higher concentrations (10^{-8} to 10^{-7} M), estradiol no longer increases AVP expression. This may in part reflect the activation of ER β , which compensates the transcriptional action of ER α . Our findings are in agreement with previous studies showing that estradiol induces transcriptional activation of the AVP gene in SK-N-SH cells transfected with ER α , whereas the hormone reduces transcriptional activation of the AVP gene in SK-N-SH cells transfected with ER β (52). Although the stimulatory effect of ER α on the AVP promoter involves estrogen response elements, the action of ER β at activator protein-1 sites (52, 53) may be involved in the inhibitory effect of ER β on the AVP promoter.

The different effect of ER α and ER β on AVP expression is of physiological relevance. For instance, estradiol decreases AVP transcript levels in male mice PVN, a region in which the main ER form is ER β , and this action is abolished in ER β knockout mice (25). Our present findings, showing that the activation of ER β decreases AVP mRNA levels, are in agreement with this observation. Our results are also relevant for the estrogenic regulation of AVP in other brain regions such as the bed nucleus of the stria terminalis, the medial nucleus of the amygdala, and the periventricular preoptic nucleus, in which both ER α and ER β are expressed (54).

In male and female rodents, the in vivo expression of AVP is down-regulated by gonadectomy and up-regulated by T in the bed nucleus of the stria terminalis and medial amygdala (28, 55–57). Our present findings indicate that T up-regulates AVP expression in SH-SY5Y cells. SH-SY5Y cells express the enzyme 5 α -reductase and are therefore able to convert T in the AR agonist 5 α -DHT (44). Although we have not been able to detect AR mRNA in our cells, a previous study has reported the expression of

AR in the SH-SY5Y cell line (45). Therefore, to fully exclude the possible implication of AR in the action of T, we used the 5 α -reductase inhibitor finasteride, the AR antagonist flutamide, and the AR agonist 5 α -DHT. The effect of T on AVP expression was not affected by these treatments, indicating that AR is not involved in the regulation of AVP expression by T in the SH-SY5Y cells.

SH-SY5Y cells express aromatase (42); therefore, T may be converted to estradiol in these cells. Interestingly, the effect of T on AVP expression in SH-SY5Y cells was blocked by an aromatase inhibitor and by an ER α antagonist. This suggests that T regulates AVP expression in SH-SY5Y cells by its conversion to estradiol and the activation of ER α . In contrast to T, 5 α -DHT decreased AVP expression in SH-SY5Y cells. This effect of 5 α -DHT was not affected by flutamide and therefore seems to be independent from AR. It is known that 5 α -DHT can be converted to the androgen metabolite 3 β -diol, which is an agonist of ER β (58–61). Interestingly, the ER β agonist DPN had a similar effect to 5 α -DHT in reducing AVP expression. Therefore, one of the possible mechanisms involved in the regulation of AVP expression by 5 α -DHT is the conversion to the androgen metabolite 3 β -diol and the activation of ER β (39, 40). Indeed, we detected that 3 β -diol decreased AVP mRNA levels in SH-SY5Y cells.

A question raised by our findings is the interpretation of the results obtained with the ER antagonist ICI 182,780. In the cells treated with estradiol or T and ICI 182,780, AVP mRNA levels were not different from control values. This is in contrast with the effects of 10^{−7} M MPP and 10^{−7} M PHTPP in the presence of estradiol or T, decreasing and increasing AVP expression, respectively. One possible interpretation is that ICI 182,780 blocked the ER α -induced increase and the ER β -induced decrease in AVP mRNA levels, resulting in a net zero effect. This suggests that in other systems the differential regulation by ER α and ER β may not be fully appreciated by using ICI 182,780.

In summary, our findings indicate that estradiol regulates AVP expression in the SH-SY5Y cells through both ERs, exerting a stimulatory action via ER α and an inhibitory action via ER β . In addition, the regulation of AVP expression in the SH-SY5Y cells by T is independent of AR and involves, as for estradiol, a stimulatory action via ER α and an inhibitory action via ER β . These data suggest that distinct AVP neuronal populations coexpressing different relative levels of ER α and ER β may be differentially regulated by gonadal hormones.

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